CUSTOMER NO. 000042131 **Docket No. 170.002**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Wei He and Wei Weng

SERIAL NO.: 10/768,350 **Group Art Unit:** 1632

FILED: January 30, 2004 **Examiner: Joanne Hama**

FOR: Genetic Modification of C57 Mice

DECLARATION OF Dr. Wei He PURSUANT TO 37 C.F.R. 1.132(a)

1. I am the co-inventor in the above referenced application. I am currently a Research Scientist at Ingenious Targeting Laboratory. Inc. located at 25 E. Loop Road in Stony Brook, New York.

2. The Office Action rejected claims 1 and 2 under 35 USC sec 112 first paragraph.

Response: We have described in detail the methodology on how to produce the cells lines listed in claims 1 and 2, so than one skilled in the art will be able to follow our disclosure and produce them. In addition, we have deposited the cell lines with the American Type Culture Collection, P.O.Box 1549, Manssas, VA 201108.

3. The Office Action rejected claims 1 and 2 under 35 U.S.C sec 102(b) as being anticipated by Tarrant et al.

Response:

We have narrowed our claims 1 and 2 to C57 mouse cell lines. We believe our method will work in other non-human vertebrate animal cell lines.

4. The Office Action rejected:
   - Claim 3 as being anticipated by Schuster-Gossler et al.
   - Claims 4 and 5 as being anticipated by Couthier et al
   - Claims 1-11 as being obvious over Schuster-Gossler et al in view of Wei.

Response:

a) We have amended claims 1-8 and cancelled claims 9-11. We would like to clarify that C57BL/6J (black B6) knockout mouse technology is drawing much attention from the researchers. Scientists are struggling to improve the efficiency, especially of the ES cells and embryo combinations which affect the final step of germline transmission in the knockout process.

b) As pointed out in the Action, traditionally coat color has been used to determine the percentage of the injected ES cells contributing the host blastocysts ("chimerism of chimeras").

c) In our invention, we have used a new approach for identifying how the ES cells contribute to the host blastocysts. With this new approach we can identify the chimerism of the chimeras when we inject black B6 ES cells into black B6 blastocysts ("black into black") (our previous claims 6 and 9). This approach of "black into black" has more advantages over the method of Schuster-Gossler et al's ("black into white") because Schuster-Gossler et al's method will introduce the tyrosinase mutation, and the blastocyst production is low in white B6 strain (See pg 1026, the last paragraph).

d) The white (albino) B6 ES cell line(from C577BL/6J-Tyr c-2J mouse strain) has not been isolated or published. Injecting white B6 ES cells into black B6 blastocysts results in a "white into black" combination (our previous claim 8 and 10). Coat color can be used to identify the chimerism of chimeras. Moreover, the mouse colony is set up similar to that set up using 129 ES cells. Most importantly black B6 mice generate more embryos than their white counterparts.

e) Schuster-Gossler et al., in their model, just injected the wild type black B6 ES cells into white (albino) B6 blastocysts. That is, no genetic modification was done with black B6 ES cells. In contrast, in the present invention, we introduced genetic modification in the black ES cells first, and then injected these ES cells into white B6 blastocysts. This difference between Schuster-Gossler et al's teachings and our invention in the "black into white" protocol, clearly distinguishes our invention on their paper. (Our claim 4).

I hereby declare that all statements made herein to my knowledge are true, and all statements made on information and beliefs are believed to be true; and further, that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon.

BY: _____

Wei He, Ph.D

DATE: 1/17/05

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is transmitted by Express Class No. ED 096541875 US under 37 C.F.R. 1.10 on January 18, 2005 addressed to: Commissioner for Patents, Alexandria, VA 22313-1450.

Rashida A. Karmali
Attorney for Applicants

Rashida A. Karmali
Signature

1/18/2005

BUDAPEST TREATY DEPOSIT FORM (BP/1)

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108

TO DEPOSIT OR TO CONVERT A DEPOSIT TO MEET THE REQUIREMENTS OF THE BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF A PATENT PROCEDURE

ALL QUESTIONS MUST BE COMPLETED IN ENGLISH. PLEASE USE ONE FORM FOR EACH STRAIN DEPOSITED.

1. Name of deposit. Please mark the appropriate box and provide the information requested for the material:

   ☐ **Microorganism** – the complete scientific name including genus and species plus the source of the material
   ☐ **Virus** – the name, whether plant or animal, and source including geographic location
   ☒ **Cell line** – the species and tissue of origin, geographical source of isolation, and any known associated hazards (HIV, EBV, etc.)
   ☐ **Genetic material** – the name of organism from which vector, clone or library is derived, the source of the DNA insert identified by species (e.g., human, mouse) or scientific name, the name of gene, and the identity of the host organism
   ☐ **Consortia or mixed culture** – the identity of each component of the mixture
   ☐ **Seeds, embryos, insect eggs, etc.** – the common name, the scientific name of the source of the deposit, and geographical source

   IC1 : C57 BL/6 , from the mouse embryo.

2. Strain designation* (i.e., number, symbols, etc). Vial #1 ~ #25
   ***The strain designation must correspond with the vial labels.**

3. Is this an original deposit under the Budapest Treaty? ☒ Yes ☐ No

4. Is this a request for a conversion of a deposit already at the ATCC to meet the requirements of the Budapest Treaty?
   ☐ Yes ☒ No
   If yes, please indicate ATCC designation. _____

5. Is this deposit a mixture of microorganisms or cells? ☐ Yes ☒ No
   If yes, please describe:
   _____

6. Provide details necessary to cultivate, test for viability and store the deposit. If a mixture, provide description of components and a method to check for presence. If a plasmid, provide name of host and antibiotic resistance.
   You can use standard ES culture medium from GibCo to culture them and test the for Viability. You also can use standard mammalian cells freezing method to freeze them down and store them in −80°C freezer.

7. Provide sufficient description so that ATCC may confirm deposit properties (e.g., Gram negative rod).
   When IC1 ES cells are injected into albino B6 blastocysts, Chimeras should be seen.

   a. If deposit is a cell culture, is it being cultured in the presence of antibiotics? ☒ Yes ☐ No
      If yes, please list the antibiotics: pen / strep

   b. If deposit is a hybridoma, what is the isotype of the antibody produced? _____

8. Safety: Is this strain hazardous to humans? No Animals? No Plants? No

   If yes, what is the recommended biosafety level for working with this strain? _____
   (Refer to *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control. Washington, DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.)

9. Regulatory Compliance:

a. Was the material derived from a human? ☐ Yes ☒ No
If yes, was an IRB-approved consent form (human subjects) obtained? ☐ Yes ☐ No

b. Was this material obtained from wildlife? ☒ Yes ☐ No
If yes, please indicate genus and species and whether wild or captive bred. Mus Musculus

c. Is work performed at your facility with exotic viruses affecting livestock and avian species? ☐ Yes ☒ No

d. Identify any reagents of animal origin used to cultivate this organism/cell line (serum, growth factors, trypsin, etc.) and manufacturer, if known: Serum (Fetal Bovine Serum from Hyclone)
growth factor (LIF from chemicon)

10. Availability:

Prior to issuance of a U.S. Patent, ATCC will only make a culture available as instructed by the depositor or relevant patent office. Samples must be provided to a specific investigator if a pertinent Patent Office under the Budapest Treaty instructs ATCC to do so. The following questions **must** be answered:

a. As of date of deposit or conversion to meet the requirements of the Budapest Treaty, do you wish the deposit to be made available to anyone who requests a culture? If yes, there are no restrictions on distribution. **Answering no will ensure the deposit is not available until the patent has issued.** ☐ Yes ☒ No

b. As of the date of deposit or conversion to meet the requirements of the Budapest Treaty, do you wish the deposit to be made available to requesters that satisfy Patent Offices in countries **not** signatory to the Budapest Treaty? ☐ Yes ☒ No
If "yes," please state which countries: _____

Please note that if you are converting your deposit to meet the requirements of the Budapest Treaty, and your deposit has already been released for distribution due to the issuance of a U.S. Patent, you **cannot** restrict it from further distribution. After a U.S. Patent issues and we are so notified, ATCC makes the culture available to anyone who requests it, as allowed under U.S. Patent and Trademark Office (USPTO) Rules and Regulations (37 CFR 1.808 [a][2]).

11. Notification: ATCC will notify you of your ATCC number after viability of the deposit has been confirmed.

Name of individual to notify: Wei He

Fax: 631-444-6645 Phone: 631-444-6640 E-mail: whe@genetargeting.com

12. Payment by check or credit card (MasterCard, VISA or American Express) must accompany the deposit unless prior arrangements for billing have been made and approved. ATCC accepts purchase orders for the exact amount.

Purchase Order No. _____ Check No. _____

Credit Card number. _____ ☐ MasterCard ☐ VISA ☐ American Express

Exp. Date: _____ Name shown on card: _____
(Please print clearly or type)

Signature of card holder _____

PAYMENT: ATCC MUST HAVE A BILLING ADDRESS, CONTACT PERSON, PHONE AND FAX FOR ALL DEPOSITS:

Contact Name: Ellen Chen

Billing Address: 25 Health Sciences Dr.
Stony Brook, NY 11790

Phone: (631) 444-6640 Fax: (631) 444-6645

Do you have a current ATCC account number? ☐ Yes ☒ No

If Yes: ATCC Account Number = _____

If No: To apply for an account with ATCC, please complete a New Account Application located on our Web site (www.atcc.org) and return it with supporting documentation to ATCC for approval.

13. Name, address, phone and fax number of your Attorney of Record.

Rashida A. Karmali
99 Wall Street, 13th Fl., New York, NY 10005
Tel: 212-659-9653 Fax: 212-651-9654 (Ref: Docket or Case No. _____)

14. **MUST BE COMPLETED.** Deposited on behalf of: (Verify with your management who owns the deposit. The owner is usually a company or institution, and not an individual.)

inGenious Targeting Laboratory Inc

I understand and agree that the deposit may not be withdrawn by me for the period specified in Rule 9.1 of the Budapest Treaty (at least 30 years after the date of deposit or 5 years after the date of the most recent request for the deposit, whichever is longer), and that if a culture should die or be destroyed during the life of the patent, or the period of time so specified, it is my responsibility to replace it with a living culture of the same organism or cell. In the cases of viruses, cell cultures, plasmids, embryos, and seeds, it is my responsibility to supply a sufficient quantity for distribution for the period of time specified above.

Wei He	qwene	1/17/05
Printed Name	Signature	Date

Address. 25 Health Sciences Drive, Suite 105

Phone: (631) 444 6640 Fax (631) 444 6645 E-mail: whe@genetargeting.com

SHIPPING INFORMATION

BEFORE SHIPPING, PLEASE CONTACT THE ATCC PATENT DEPOSITORY FOR SHIPMENT ADVICE:

Fax: (703) 365-2745
E-mail: PatentDeposit@atcc.org

SHIPPING NOTICE:

The depositor is ultimately responsible for the shipment of deposits to ATCC and compliance with all applicable government regulations for the packaging and movement of the material. The depositor shall indemnify ATCC, to the extent permitted by law, against claims resulting from the violation of applicable government regulations caused by the depositor's shipment of deposits to ATCC.

STORAGE & FEES

Storage: Cultures are stored for 30 years from date of deposit or five years after the last request for a sample, whichever is longer, as required under the rules of patent offices in most countries.

Fees: All fees are subject to change. For current fees and other information, check our Web site at www.atcc.org or request a quotation of fees by e-mail at PatentDeposit@atcc.org or fax: (703) 365-2745.

ATCC USE ONLY: ATCC DESIGNATION_____ **REC'D** _____ **V.T. RESULT**_____

BUDAPEST TREATY DEPOSIT FORM (BP/1)

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P.O. Box 1549
Manassas, VA 20108

TO DEPOSIT OR TO CONVERT A DEPOSIT TO MEET THE REQUIREMENTS OF THE BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF A PATENT PROCEDURE

ALL QUESTIONS MUST BE COMPLETED IN ENGLISH. PLEASE USE ONE FORM FOR EACH STRAIN DEPOSITED.

1. Name of deposit. Please mark the appropriate box and provide the information requested for the material:

   Microorganism – the complete scientific name including genus and species plus the source of the material
   Virus – the name, whether plant or animal, and source including geographic location
   X **Cell line** – the species and tissue of origin, geographical source of isolation, and any known associated hazards (HIV, EBV, etc.)
   Genetic material – the name of organism from which vector, clone or library is derived, the source of the DNA insert identified by species (e.g., human, mouse) or scientific name, the name of gene, and the identity of the host organism
   Consortia or mixed culture – the identity of each component of the mixture
   Seeds, embryos, insect eggs, etc. – the common name, the scientific name of the source of the deposit, and geographical source

   The cell line to be deposited is called IAC1. It's from C57 BL/6J-Tyr^{C-2J} mouse strain (Mus Musculus). We bought this kind of mouse from Jackson lab. It's derived from mouse embryos.

2. Strain designation* (i.e., number, symbols, etc). Vial#1 ~ #25 labeled with IAC1
   ***The strain designation must correspond with the vial labels.**

3. Is this an original deposit under the Budapest Treaty? X Yes No

4. Is this a request for a conversion of a deposit already at the ATCC to meet the requirements of the Budapest Treaty?
   Yes X No
   If yes, please indicate ATCC designation. _____

5. Is this deposit a mixture of microorganisms or cells? Yes X No
   If yes, please describe:
   _____

6. Provide details necessary to cultivate, test for viability and store the deposit. If a mixture, provide description of components and a method to check for presence. If a plasmid, provide name of host and antibiotic resistance.

   You can use standard ES culture medium from Gibco to culture IAC1 ES cells and test for viability. You also can use standard mammalian cell freezing method to freeze them down and store them in −80°C freezer.

7. Provide sufficient description so that ATCC may confirm deposit properties (e.g., Gram negative rod).

   When IAC1 ES cells are injected into black B6 blastocysts, Chimeras (White on black background) should be seen

   a. If deposit is a cell culture, is it being cultured in the presence of antibiotics? X Yes No
      If yes, please list the antibiotics: pen / strep

   b. If deposit is a hybridoma, what is the isotype of the antibody produced? _____

8. Safety: Is this strain hazardous to humans? No Animals? No Plants? No

   If yes, what is the recommended biosafety level for working with this strain? _____
   (Refer to *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control. Washington, DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.)

9. Regulatory Compliance:

a. Was the material derived from a human? ☐ Yes ☒ No
If yes, was an IRB-approved consent form (human subjects) obtained? ☐ Yes ☐ No

b. Was this material obtained from wildlife? ☒ Yes ☐ No
If yes, please indicate genus and species and whether wild or captive bred. Mus Musculus

c. Is work performed at your facility with exotic viruses affecting livestock and avian species? ☐ Yes ☒ No

d. Identify any reagents of animal origin used to cultivate this organism/cell line (serum, growth factors, trypsin, etc.) and manufacturer, if known: Serum (Fetal Bovine Serum from Hyclone)
growth factor (LIF from chemicon)

10. Availability:

Prior to issuance of a U.S. Patent, ATCC will only make a culture available as instructed by the depositor or relevant patent office. Samples must be provided to a specific investigator if a pertinent Patent Office under the Budapest Treaty instructs ATCC to do so. The following questions **must** be answered:

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If "yes," please state which countries: _____

Please note that if you are converting your deposit to meet the requirements of the Budapest Treaty, and your deposit has already been released for distribution due to the issuance of a U.S. Patent, you **cannot** restrict it from further distribution. After a U.S. Patent issues and we are so notified, ATCC makes the culture available to anyone who requests it, as allowed under U.S. Patent and Trademark Office (USPTO) Rules and Regulations (37 CFR 1.808 [a][2]).

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Purchase Order No. _____ Check No. _____

Credit Card number. _____ ☐ MasterCard ☐ VISA ☐ American Express

Exp. Date: _____ Name shown on card: _____
(Please print clearly or type)

Signature of card holder _____

PAYMENT: ATCC MUST HAVE A BILLING ADDRESS, CONTACT PERSON, PHONE AND FAX FOR ALL DEPOSITS:

Contact Name: Ellen Chen

Billing Address: 25 Health Sciences Dr.
Stony Brook NY 11790

Phone: (631) 444-6640 Fax (631) 444-6645

Do you have a current ATCC account number? ☐ Yes ☒ No

If Yes: ATCC Account Number = _____

If No: To apply for an account with ATCC, please complete a New Account Application located on our Web site (www.atcc.org) and return it with supporting documentation to ATCC for approval.

13. Name, address, phone and fax number of your Attorney of Record.

Rashida A. Karmali
99 Wall Street, 13th Fl., New York, NY 10005
Tel: 212-659-9653 Fax: 212-651-9654 (Ref: Docket or Case No. _____)

14. **MUST BE COMPLETED.** Deposited on behalf of: (Verify with your management who owns the deposit. The owner is usually a company or institution, and not an individual.)

InGenious Targeting Laboratory Inc

I understand and agree that the deposit may not be withdrawn by me for the period specified in Rule 9.1 of the Budapest Treaty (at least 30 years after the date of deposit or 5 years after the date of the most recent request for the deposit, whichever is longer), and that if a culture should die or be destroyed during the life of the patent, or the period of time so specified, it is my responsibility to replace it with a living culture of the same organism or cell. In the cases of viruses, cell cultures, plasmids, embryos, and seeds, it is my responsibility to supply a sufficient quantity for distribution for the period of time specified above.

Wei He	qwerne	1/17/05
Printed Name	Signature	Date

Address. 25 Health Sciences Drive, Suite 105

Phone: (631) 444 6640 Fax (631) 444 6645 E-mail: whe@genetargeting.com

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Fax: (703) 365-2745
E-mail: PatentDeposit@atcc.org

SHIPPING NOTICE:

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STORAGE & FEES

Storage: Cultures are stored for 30 years from date of deposit or five years after the last request for a sample, whichever is longer, as required under the rules of patent offices in most countries.

Fees: All fees are subject to change. For current fees and other information, check our Web site at www.atcc.org or request a quotation of fees by e-mail at PatentDeposit@atcc.org or fax: (703) 365-2745.

ATCC USE ONLY: ATCC DESIGNATION_____ **REC'D** _____ **V.T. RESULT**_____